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Award Number: W81XWH-05-1-0195

TITLE: Analysis of Learning Disabilities of Tuberous Sclerosis Complex in Drosophila

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REPORT DATE: January 2006

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-01-2006	Final	30 Dec 2004 – 29 Dec 2005

4. TITLE AND SUBTITLE

Analysis of Learning Disabilities of Tuberous Sclerosis Complex in Drosophila

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-1-0195

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Cold Spring Harbor Laboratory
Cold Spring Harbor, NY 11724

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Intellectual impairments/learning disabilities are among the most frequent clinical manifestations of tuberous sclerosis complex (TSC). In this proposal, we explored Drosophila models for TSC related learning defects. Both tsc1 and tsc2 genes are conserved in flies and mutants are available. Our preliminary data indicated that mutations in both genes were able to cause learning defects even in heterozygous mutants. We also showed that this gene could be critically involved in learning processes for over expression of both gene together led to an increase in memory retention. Although these studies were still very preliminary, it is encouraging that Drosophila may serve as models for gaining insights into molecular basis of the TSC disorder-related cognitive dysfunction.

15. SUBJECT TERMS
No subject terms provided.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	7	**19b. TELEPHONE NUMBER** *(include area code)*

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INTRODUCTION

Intellectual impairments/learning disabilities are among the most frequent clinical manifestations of tuberous sclerosis complex (TSC). More than 50% of TSC individuals have reduced IQ of less than 70, while the rest have reduced average IQ than the normal population and are prone to specific cognitive difficulties. MRI studies have suggested a correlation between then umber of cortical tubers and the neurological abnormalities, including epilepsy, autism and intellectual disabilities. Analyses of TSC1 knockout mouse suggest that the epilepsy may be resulted from abnormal function of astrocytes, because astrocyte-specific *TSC1* knockout mouse causes progressive epilepsy and impaired glutamate transporter expression and function. In comparison, our understanding of the molecular basis of learning disabilities in TSC is very limited. In this proposal, we wanted to examine whether Drosophila can be a model for studying molecular basis of the learning defects observed in TSC patients. Specifically, we have examined learning and memory in Drosophila TSC1 and TSC2 mutants.

BODY

Aim1. Examining the effect of *tsc1* and *tsc2* mutations on olfactory learning in Drosophila. All *tsc1* and *tsc2* mutants we have obtained, $tsc1_{460}$ and $gigas_{109}$ (encoding TSC2), are both lethal in homozygous. Therefore, we were only able to examine behaviors of heterozygous mutant flies (tsc1/+ or gigas/+) or heteroallelic flies (tsc1/gigas), which were obtained through crosses of tsc1/TM3 or gigas/TM3 with 2202U (isogenic line with white eyes), or tsc1/TM3 with gigas/TM3. Since TM3 contains Ser, which has a wing phenotype, flies with normal wings were selected. We have used the well- established odor and foot shock associative learning paradigm to test learning in these mutants. Briefly, about 150 flies were loaded into training chamber, in where flies subjected to odor A paired with electric shock followed by odor B without shock. After this training cycle, flies were moved to a T maze choice point exposed to odors A and B simultaneously from opposite direction. Normal flies learn to avoid odor A that pairs with electric shock. We have found that learning scores for $tsc1_{460}$/+ were close to the control, but $gigas_{109}$/+ was significantly lower than control (Fig. 1in Appendix). It is likely that $tsc1_{460}$/+ was too weak to exert significant effects. Indeed, learning became worse in double heterozygous mutants $tsc1_{460}/gigas_{109}$. This result suggests that TSC1 and TSC2 complex may be important for learning and memory in Drosophila.

Future Experiments: Since *tsc1* heterozygous mutant flies were normal in learning, we plan to make homozygous mutant flies carrying a inducible normal tsc1 gene, $hs\text{-}tsc1^{+}$/+;$tsc1^{460}$/ $tsc1^{460}$ through a cross of $tsc1^{460}/TM3$ x $hs\text{-}tsc1^{+}/hs\text{-}tsc1^{+}$; $tsc1^{460}/TM3$ for behavioral tests. During development, we are going to make 30 minutes heat shock every 12h at 37ºC. Homozygous mutant flies will survive to adult. Then heat shock will be stopped for 2 days and then learning will be tested in these homozygous mutant flies. These mutants were confirmed by the lethal phenotypes and the induced expression was indicated genetically for its effect in rescuing lethality of corresponding mutations.

Aim2. Rescuing the learning defect with *tsc* transgenes, therefore determining whether the learning defect is due to developmental abnormalities or not. We were unable to examine this part of proposed experiments. We have made appropriate flies through combination, but later realized that flies were contaminated and could not be used for examination. We have just received newly requested flies and are making necessary genetic crosses.

Aim3. Testing the effect of overexpression of *tsc* genes on learning. Flies carrying double transgenes hs-tsc1$_+$/hs-tsc2$_+$ (indicating hetero-transgene for both transgenes were on the second chromosome) were examined before and after subject to heat-shock treatment. With heat shock (30 minutes at 37 $_o$C and then resting for 3 hours before training), there was not significant difference for learning scores between these two groups of flies. However, when we examined 3 hour retention, memory scores [measured by performance index (PI), see figure 1 legends for details] for those subjecting to heat-shock treatment appeared to be higher [PI=45±4 (n=8) for with heat shock; PI=34±5 (n=7) for without heat shock]. This suggests that overexpression of TSC1 and 2 together may be able to improve memory.

Future Experiments: It is important to determine whether overexpression of TSCs is able to improving memory. Such observation will be critical for gaining an understanding of how TSCs are involved in learning and memory. Although statistically significant, the difference observed here is very limited. We will explore effects of different regimens of heat shock and resting time and hope to find the optimal conditions for studying the enhanced memory effects.

KEY RESEARCH ACCOMPLISHMENTS

1. Our preliminary data indicated that learning was defective in gigas/+ and tsc1/gigas mutant flies but remained normal in tsc1/+ heterozygous mutant flies.
2. We found that overexpression of tsc1 and tsc2 genes together improved 3 hour memory.

REPORTABLE OUTCOMES

None

CONCLUSIONS

This is a report for an idea award. We explored whether learning is affected in tsc1 or tsc2 fly mutants. The reported data is very preliminary for we were only able to examine heterozygous mutants and learning phenotypes were mild. However, this conclusion is supported by observation that expression of TSC1 or TSC2 together appears to enhance memory. We were encouraged by these observations and will make further effort in determining that whether Drosophila can be a model system for gaining insights into molecular basis of TSC-dependent learning defects in patients.

Two of three tasks proposed were completed and all work reported was within the scope of the Statement of Work. Task 2 was not completed because it was found that the transgenic flies group was contaminated. We are beginning anew with fresh flies.

PERSONNEL

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Yalin Wang

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Appendix

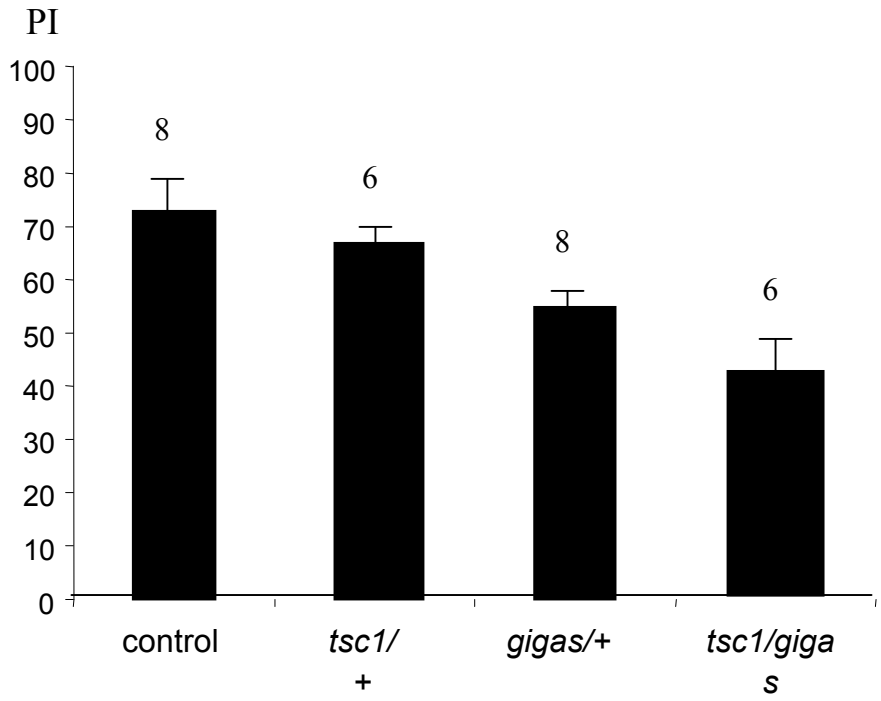

Figure 1, Effects of mutations at tsc1 and tsc2 (gigas) on learning.
PI (performance index)=100% X (number of flies move towards odorB - flies towards odorA)/(number of flies towards odorB +flies towards odorA). Control flies were isogenic lines 2202U with white eyes. The number of PIs for each genotypes are indicated. The PI for gigas/+ is just significant in t-test (<0.05) and tsc1/gigas is more significant (<0.01) when compared to PIs in the control. The procedure for learning test is described briefly in the text and for details see ref (Guo et al., 1997).